SUPPORTING INFORMATION

Design of subdomain 2 and 3 and purification of subdomains

Subdomain 2 is designed from aa R185 to A324 of ECD containing sites 2 and 3 in lobe 2, and subdomain 3 (aa R323 to L494) contains sites 4 and 5. The designed subdomain proteins were expressed as $(His)_6$-tag fusion proteins in Escherichia coli BL21(DE3)pLysS, Rosetta(DE3) or Tuner(DE3)pLacI and purified using affinity chromatography of the Hitrap Ni^{2+}-chelating column (GE Healthcare). As shown in Fig. S1, all these proteins were successfully purified to near homogeneity with over 90% purity.

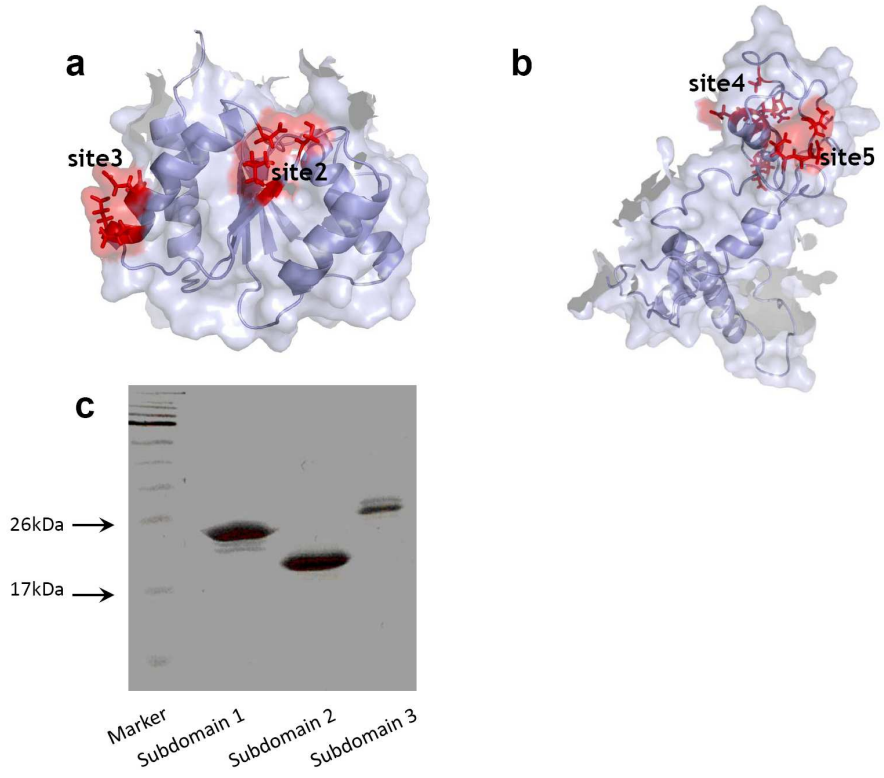

Fig. S1. Design of subdomains 2 and 3 and SDS-PAGE of purified proteins. (a) Model structure of subdomain 2 (R185-A324) which contains two putative Ca2+-binding sites (site 2 and site 3). (b) Model structure of subdomain 3 (A323-G494) which contains site 4 and site5. (c) SDS-PAGE of purified designed subdomains. The molecular weights for subdomains 1, 2 and 3 are 22.9, 20.8 and 23.6 kDa, respectively.

Subdomains are folded

The near UV CD spectra of subdomain 1 showed significant bands in regions corresponding to immobilized aromatic residues (280-300 nm) (Fig. S2a). In addition, Ca^{2+} induced tertiary structural changes in subdomain 1, as suggested by substantially more prominent near UV CD bands around 275 nm and/or 288 nm (Fig. S2a). To further estimate the overall solvent accessibility of the tryptophan residues within each subdomain, we performed additional fluorescence quenching studies with acrylamide. As shown in Fig. S2b and table S1, the fully exposed free L-tryptophan has a K_{sv} of 20.9 M^{-1}, whereas the apparent collisional quenching constants K_{sv} for subdomains 1, 2 and 3 are 3.7, 13.4, and 3.8 M^{-1}, respectively. All these results suggest that the aromatic residues in these three subdomains were at least partially buried, as in other folded proteins.

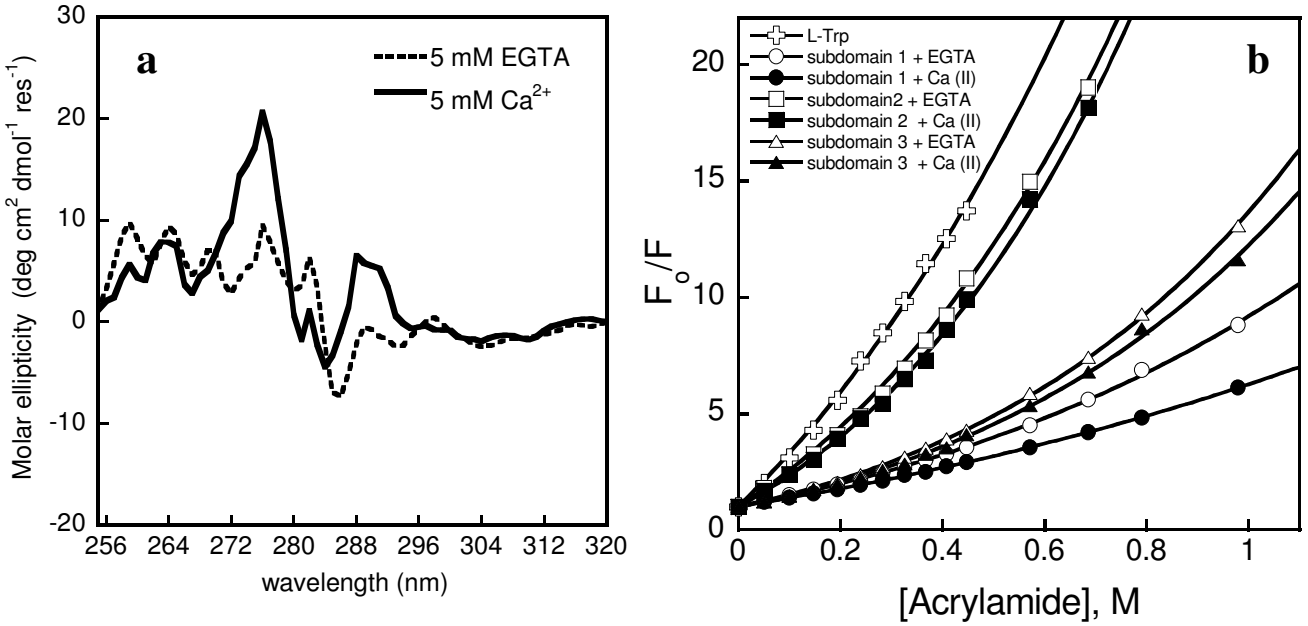

Fig. S2. (a) Near UV CD spectra of subdomains with 5 mM EGTA (dashed line) or 5 mM Ca^{2+} (solid line). (b) Stern-Volmer plots of Trp fluorescence quenching for subdomain 1 (circle), subdomain 2 (square) and subdomain 3 (triangle) in the absence (open symbols) and presence (closed symbols) of 5 mM Ca^{2+}. All the buffers consist of 50 mM Tris, 135 mM NaCl, 10 mM KCl, pH 7.4. L-tryptophan (L-Trp) in the same buffer is used here as control.

Table S1. Stern-Volmer constants for subdomains and L-Trp. The quenching constants were obtained by fitting data with Eq. 4.

Sample	K_{sv}		V	
	EGTA	Ca	EGTA	Ca
L-Trp	20.9 ± 0.9	21.3 ± 0.5	0.67 ± 0.10	0.66 ± 0.30
Subdomain 1	3.7 ± 0.1	3.2 ± 0.1	0.67 ± 0.03	0.40 ± 0.01
Subdomain 2	13.4 ± 0.6	11.1 ± 0.2	0.94 ± 0.05	1.09 ± 0.02
Subdomain 3	3.8 ± 0.1	3.5 ± 0.2	1.04 ± 0.02	1.00 ± 0.05

Multiple Metal-Binding Processes

Fig S3 shows the resonances corresponding to protons from methyl groups in subdomain 1. This region experienced changes in sudden peak shape and the appearance of more dispersed peaks when the Ca^{2+} concentration reaches ~2 mM.

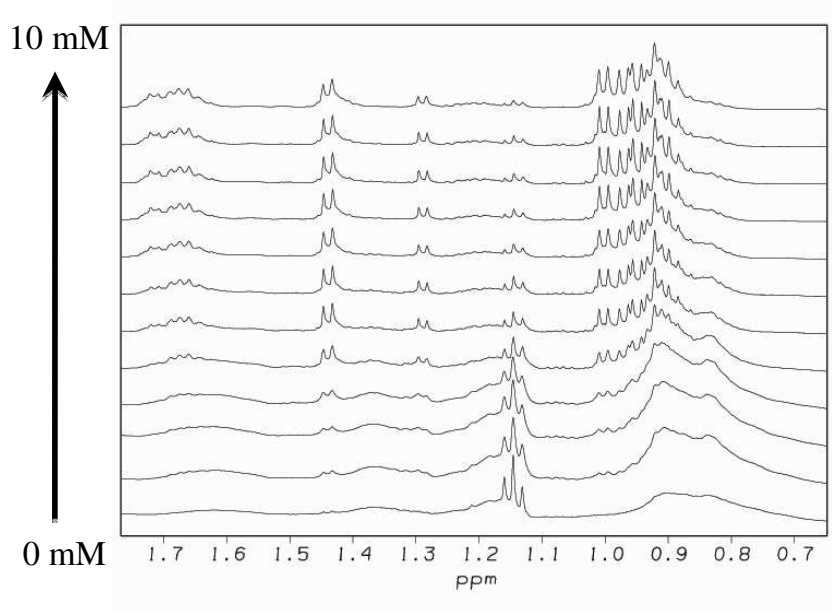

Fig. S3. Ca^{2+} titration of subdomain 1 monitored by 1D ^{1}H NMR. The Ca^{2+} concentrations from bottom to up are: 0, 0.1, 0.3, 0.7, 1.1, 1.5, 1.9, 2.8, 4.7, and 6.7, 8.6 and 11.5 mM.